The limitation of the patch assay is that the *de novo* hairs form a cyst beneath the skin and do not penetrate to the surface. Therefore, it is hard to see hair growth without a surgical biopsy. In addition, the orientation of hair follicles generated in the patch assay is generally random, and the extrafollicular dermal macroenvironment is not restored.

To be useful, engineered hairs should have organized follicular architecture, hair differentiation products, and proper planar arrangement, as well as be able to cycle and regenerate (Chuong *et al.*, 2007). To evaluate each of the above properties, good models should be efficient and reasonably easy to use, so they can be used for high-throughput screening. There may not be one ideal fully functional model of hair reconstitution. Complete hair reengineering could require a combination of assays to assess each aspect of hair morphogenesis. It took nature millions of years to evolve the hair follicle (Wu *et al.*, 2004) and it will take humans many years to learn how to engineer the hair follicle and build complex tissues properly. HFM has resulted in strides in the right direction, allowing more efficient screening of drugs and small molecules for complex tissue interactions. Future endeavors should also focus on the development of more realistic and cosmetically acceptable models to allow clinical translation into curing alopecia and other hair disorders. Brick by brick and stone by stone, we will continue to advance toward the ultimate goal of fully functional engineered skin.

## CONFLICT OF INTEREST

The authors state no conflict of interest.

## ACKNOWLEDGMENTS

We thank Warren Garner for discussion. L.F.L. is supported by 5 F32 GM082019-02. C.-M.C. is supported by NIAMS AR 42177 and AR 47364.

## REFERENCES

Blanpain C, Lowry W, Geoghegan A, Polak L, Fuchs E (2004) Self-renewal, multipotency, and the existence of two cell populations within an epithelial stem cell niche. *Cell* 118:635–48

Chuong C, Cotsarelis G, Stenn K (2007) Defining hair follicles in the age of stem cell bioengineering. *J Invest Dermatol* 127:2098–100

Dhouailly D, Rogers G, Sengel P (1978) The specification of feather and scale protein synthesis in epidermal–dermal recombinations. *Dev Biol* 65:58–68

Ehama R, Ishimatsu-Tsuji Y, Iriyama S, Ideta R, Soma T, Yano K *et al.* (2007) Hair follicle regeneration using grafted rodent and human cells. *J Invest Dermatol* 127:2106–15

Havlickova B, Bíró T, Mescalchin A, Arenberger P, Paus R (2004) Towards optimization of an organotypic assay system that imitates human hair follicle-like epithelial–mesenchymal interactions. *Br J Dermatol* 151:753–65

Havlickova B, Bíró T, Mescalchin A, Tschirschmann M, Mollenkopf H, Bettermann A *et al.* (2009) A human folliculoid microsphere assay for exploring epithelial–mesenchymal interactions in the human hair follicle. *J Invest Dermatol* 129:972–83

Morris R, Liu Y, Marles L, Yang Z, Trempus C, Li S *et al.* (2004) Capturing and profiling adult hair follicle stem cells. *Nat Biotechnol* 22:411–7

Oliver R (1967) Ectopic regeneration of whiskers in the hooded rat from implanted lengths of vibrissa follicle wall. *J Embryol Exp Morphol* 17:27–34

Philpott M, Sanders D, Kealey T (1995) Cultured human hair follicles and growth factors. *J Invest Dermatol* 104:44S–5S

Scandurro AB, Wang Q, Goodman L, Ledbetter S, Dooley TP, Yuspa SH *et al.* (1995) Immortalized rat whisker dermal papilla cells cooperate with mouse immature hair follicle buds to activate type IV procollagenases in collagen matrix coculture: correlation with ability to promote hair follicle development in nude mouse grafts. *J Invest Dermatol* 105:177–83

Weinberg W, Goodman L, George C, Morgan D, Ledbetter S, Yuspa S *et al.* (1993) Reconstitution of hair follicle development in vivo: determination of follicle formation, hair growth, and hair quality by dermal cells. *J Invest Dermatol* 100:229–36

Widelitz, R.B., Jiang, T.-X., Noveen, A., Chen, C.-W. J. and Chuong, C.-M. (1996) FGF induces new feather buds from developing avian skin. *J Invest Dermatol* 107: 797-803

Wu P, Hou L, Plikus M, Hughes M, Scehnet J, Suksaweang S *et al.* (2004) Evo-Devo of amniote integuments and appendages. *Int J Dev Biol* 48:249–70

Zheng Y, Du X, Wang W, Boucher M, Parimoo S, Stenn K (2005) Organogenesis from dissociated cells: generation of mature cycling hair follicles from skin-derived cells. *J Invest Dermatol* 124:867–76

*See related article on pg 984*

# Ectodysplasin Signaling in Cutaneous Appendage Development: Dose, Duration, and Diversity

Denis J. Headon[1]

**The development of several types of skin appendages is guided by prenatal ectodysplasin signaling. In this issue, Cui *et al.* report on the dose and duration of ectodysplasin signaling required for the maintenance and morphogenesis of different types of appendages. They report that achievement of an intimate arrangement between epithelial and mesenchymal cell populations correlates with the acquisition of autonomy from ectodysplasin stimulation.**

*Journal of Investigative Dermatology* (2009), **129**, 817–819. doi:10.1038/jid.2008.426

### Cutaneous appendage development

The skin arises from a simple sheet of embryonic ectoderm underlaid by mesenchyme. The cells in this epithelial sheet are initially homogeneously distributed, but they subsequently undergo clustering at specific locations to produce an array of placodes. Depending on their location on the body, these placodes develop into a number of diverse cutaneous appendages, including glands, teeth, and several types of hair follicles. The generation of a mature organ from the embryonic placode involves production of a downgrowth resulting from rapid epithelial

[1]*Division of Developmental Biology, Roslin Institute and R(D)SVS, University of Edinburgh, Midlothian, United Kingdom*

*Correspondence: Dr. Denis J. Headon, Division of Developmental Biology, Roslin Institute, Midlothian EH25 9PS, United Kingdom. E-mail: denis.headon@roslin.ed.ac.uk*

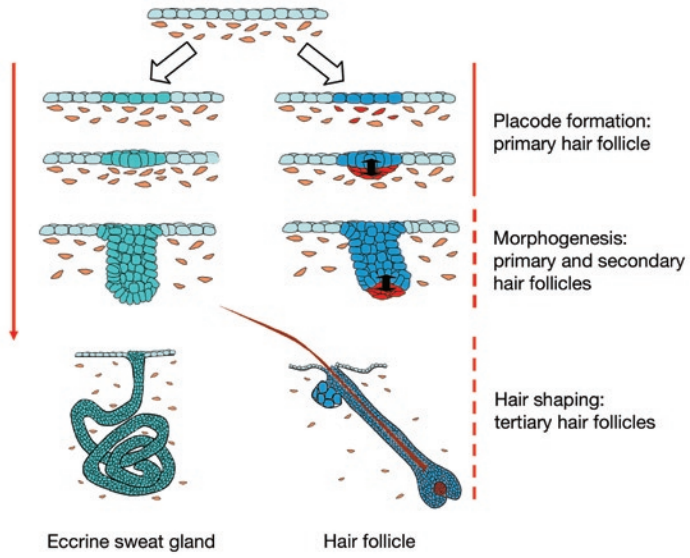


**Figure 1. The influence of ectodysplasin at different stages of ectodermal appendage development.** Acquisition of appendage cell fate is indicated by altered cell shading. Developmental stages with an absolute requirement for Eda to produce a functioning organ are indicated by a solid line, whereas an Eda influence that is not essential for organ production is indicated by a dashed line. Block arrows indicate epithelial–mesenchymal interactions in the hair follicle primordium. Formation of a dermal papilla in hair follicle development coincides with independence from Eda action. The stage in sweat gland development at which Eda withdrawal is tolerated is unknown.

proliferation followed by cellular differentiation to enable physiological functioning of the appendage (Schmidt-Ullrich and Paus, 2005).

The use of common signaling pathways to guide cell behavior underlies the similar cellular rearrangements observed during the development of multiple appendage types. Although regulatory efficiency is achieved by employing the same genes in multiple situations, one outcome of this shared genetic basis is that inherited conditions affecting appendage development tend to be syndromic, affecting multiple appendage types. One such condition, hypohidrotic ectodermal dysplasia (HED), is characterized by a reduction in hair follicle development, the growth of only a few misshapen teeth, and the absence of eccrine sweat glands.

HED is caused by mutations that affect components of a tumor necrosis factor (TNF)-like signaling pathway. Activation of this pathway is initiated by binding of the TNF-like ligand ectodysplasin (EDA) to its transmembrane receptor, EDAR. EDAR then connects to a canonical TNF signaling cascade through a dedicated adapter protein, ultimately leading to the stimulation of NF-κB. Mutation of *EDA* is the most common cause of HED, which typically presents in boys because of its presence on the X chromosome (Mikkola, 2008). Interestingly, this signaling system not only is common to a range of ectodermal appendages but is required for appendage formation across the vertebrates, from humans to fish (Harris *et al.*, 2008). The breadth of model organs and organisms available for study, together with a well-characterized signaling pathway, permit an examination of the quantity and timing of EDA action in guiding normal development.

## Critical periods and critical durations in appendage development

Cutaneous appendages form in a temporal sequence, with only one type of appendage produced in a particular skin region at a given time. For example, three distinct waves of hair follicle formation produce the three different types of hair follicles in the adult mouse. From embryonic day 14 (E14) to E16, primary hair follicles are formed; these make the long guard hairs of the coat and do not develop in animals lacking *Eda*. In contrast, the secondary and tertiary follicles initiate from approximately E16 and E19, respectively, and produce distinct hair types. These later waves of folliculogenesis do occur in *Eda* mutant mice, although abnormal hairs are produced.

The elucidation of the molecular basis of X-linked HED has enabled therapeutic efforts aimed at EDA protein replacement during development. The efficacy of such experimental therapies relies on identification of the critical periods during development at which different tissues are capable of responding to EDA. Addressing this issue in a mouse HED model, Gaide and Schneider (2003) administered a bolus of a recombinant EDA fusion protein at different times during development and then determined the degree of phenotypic rescue in adult animals. They found that the critical period at which EDA action is required to produce a particular appendage generally matches the time at which development of that structure is normally initiated. Thus, midgestational EDA administration effected a much broader rescue of mutant phenotypes than did postnatal EDA treatment (Gaide and Schneider, 2003).

Cui *et al.* (2009, this issue) have also addressed the timing of Eda action in appendage development, but from a very different perspective. Using a mouse model in which the only source of Eda is a transgene that can be switched off by administration of doxycycline, they have examined the critical duration of signaling required to stabilize the development of incipient hair follicles and sweat glands. They report that withdrawing Eda at E15, when the skin is populated with primary hair follicle placodes, leads to a lack of these follicles in adults, demonstrating the requirement for a duration of Eda signal beyond the initial stages of placode formation. Abolition of *Eda* expression at E17 or later, however, led to a normal complement of guard hairs in the adult coat. This acquisition of Eda autonomy correlates with the establishment of a dermal papilla precursor in close association with the epithelial downgrowth (Figure 1). Thus, progression of hair follicle development shifts from a reliance on the widely produced Eda signal to a more intimate reciprocal communication between the two closely juxtaposed cell populations.

Eccrine sweat glands develop slightly later than primary hair placodes and in the mouse they form only on the footpads. Despite these differences, an E19 sweat gland rudiment closely resembles an E17 hair follicle in the extent of epithelial downgrowth, but with the striking difference that there is no sign of an accompanying mesenchymal signaling center. In contrast to the hair follicle rudiment, withdrawal of the Eda signal at this stage results in a failure of gland development, suggesting that the developing sweat gland's solitary epithelial cord requires sustained ectodysplasin stimulation to reach maturity.

> EDA action is critically time dependent.

The ability to remove Eda also allows for an examination of the cellular consequences of signal starvation. Eda withdrawal from the sweat gland rudiment leaves epithelial remnants lacking a definite organ identity stranded in the dermis. It would be interesting to determine the fate of cells in the primary hair placodes that regress upon withdrawal of Eda because in normal skin all placodal cells are committed to producing hair follicles; none of these cells contributes to the interfollicular epidermis. It is possible that cells lose their commitment to a hair follicle fate and become interfollicular epidermis, although such dedifferentiation is normally observed only upon wounding (Levy *et al.*, 2005). Alternatively, these placode cells may be removed by apoptosis once starved of Eda, or perhaps they survive to find their way into the later-forming secondary and tertiary hair follicles.

**Dose effects of ectodysplasin signaling**

Although clinical studies have focused primarily on the consequences of a complete loss of *EDA* function, it is becoming clear that there is a more graded diversity in the intensity of this signal in human populations. *EDA* mutations that appear to be hypomorphic are associated with nonsyndromic tooth agenesis (Li *et al.*, 2008), and even fish tooth development appears to be particularly sensitive to reduced Edar activity (Harris *et al.*, 2008). Conversely, a variant of EDAR with a higher signal transduction potency is associated with the increased hair fiber thickness of East Asian populations (Fujimoto *et al.*, 2008; Mou *et al.*, 2008). Indeed, it is interesting to note that isolated tooth agenesis caused by *EDA* mutation (e.g., Li *et al.*, 2008) has thus far been reported in Asian families, suggesting that interaction between an *EDA* allele with reduced function and a more potent *EDAR* allele might affect the clinical presentation of HED.

In the mouse, Eda dose plays a role in determining hair fiber characteristics, with elevated *Eda* expression producing a spiky hair coat based on the angle at which hairs lay relative to the skin. The regulatable Eda model reveals that this characteristic requires a slightly longer signal than that required for primary hair follicle stabilization. However, removal of Eda prior to birth still confers a shaggy coat texture that appears to be indelible, despite the cyclical nature of hair growth in adults.

Perhaps the most complex aspect of Eda action relates to its role in shaping individual hair fibers. The majority of hairs in the mouse coat are of the zigzag type. These hairs are produced by the tertiary hair follicles and are bent at constriction sites present at intervals along the fiber. Somewhat paradoxically, loss of *Eda* function or transgenic *Eda* overexpression both have a pronounced straightening effect on these hair fibers. It has been unclear whether this straightening effect is a result of an alteration in hair follicle identity during development or due to a sustained requirement for Eda action in the mature zigzag follicle. The careful microscopic analysis reported by Cui *et al*. (2009) revealed that these straight hairs in *Eda* transgenic animals do carry regular constrictions, consistent with their possessing a true zigzag identity, but bends fail to be introduced at these sites. The insertion of constrictions requires continuous *Eda* expression during fiber growth, but Cui *et al*. (2009) imply that hair bending is an independent process requiring a very precise location, or perhaps dose, of *Eda* expression to produce the molecular asymmetries responsible for hair shaping (Hammerschmidt and Schlake, 2007).

In summary, Cui *et al*. (2009) have shown that Eda action defines appendage structure in a number of ways, acting prior to birth to enable the development of hair follicle types and then to modulate hair thickness and influence hair fiber shape in adult follicles. Thus, the dose and timing of EDA signaling have significant effects on the external phenotype, and genetic tuning of this system is likely to account for some of the extraordinary diversity of cutaneous appendages seen in humans and other vertebrates.

CONFLICT OF INTEREST

The author states no conflict of interest.

REFERENCES

Cui C-Y, Kunisada M, Esibizione D, Douglass EG, Schlessinger D (2009) Analysis of the temporal requirement for *Eda* in hair and sweat gland development. *J Invest Dermatol* 129:984–93

Fujimoto A, Kimura R, Ohashi J, Omi K, Yuliwulandari R, Batubara L *et al.* (2008) A scan for genetic determinants of human hair morphology: EDAR is associated with Asian hair thickness. *Hum Mol Genet* 17:835–43

Gaide O, Schneider P (2003) Permanent correction of an inherited ectodermal dysplasia with recombinant EDA. *Nat Med* 9:614–8

Hammerschmidt B, Schlake T (2007) Localization of Shh expression by Wnt and Eda affects axial polarity and shape of hairs. *Dev Biol* 305:246–61

Harris MP, Rohner N, Schwarz H, Perathoner S, Konstantinidis P, Nusslein-Volhard C (2008) Zebrafish eda and edar mutants reveal conserved and ancestral roles of ectodysplasin signaling in vertebrates. *PLoS Genet* 4:e1000206.

Levy V, Lindon C, Harfe BD, Morgan BA (2005) Distinct stem cell populations regenerate the follicle and interfollicular epidermis. *Dev Cell* 9:855–61

Li S, Li J, Cheng J, Zhou B, Tong X, Dong X *et al.* (2008) Non-syndromic tooth agenesis in two Chinese families associated with novel missense mutations in the TNF domain of EDA (ectodysplasin A). *PLoS ONE* 3:e2396.

Mikkola ML (2008) TNF superfamily in skin appendage development. *Cytokine Growth Factor Rev* 19:219–30

Mou C, Thomason HA, Willan PM, Clowes C, Harris WE, Drew CF *et al.* (2008) Enhanced ectodysplasin-A receptor (EDAR) signaling alters multiple fiber characteristics to produce the east Asian hair form. *Hum Mutat* 29:1405–11

Schmidt-Ullrich R, Paus R (2005) Molecular principles of hair follicle induction and morphogenesis. *Bioessays* 27:247–61